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APPLICATION NUMBER: ***60/417,738***
FILING DATE: ***October 10, 2002***
RELATED PCT APPLICATION NUMBER: *PCT/US03/28742*

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No.	ET899942517US

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Hartmut M.	Hanauske-Abel	Edgewater, New Jersey

☐ *Additional inventors are being named on the _____ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

Ligands of Eukaryotic Translation Initiation Factor 5A (eIF-5A) and Their Use in Diagnosis and Therapy of Diseases Involving Proliferation of Cells

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

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Country	US	Telephone	732-235-9350	Fax	732-235-9358

ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Specification *Number of Pages* 5
☑ Drawing(s) *Number of Sheets* 4
☐ Application Data Sheet. See 37 CFR 1.76
☐ CD(s), Number
☑ Other (specify) Claims (5 pgs); Post Card

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☑ Applicant claims small entity status. See 37 CFR 1.27.
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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☐ No.
☑ Yes, the name of the U.S. Government agency and the Government contract number are: Unknown

Respectfully submitted,

SIGNATURE MMRichardson

Date 10/07/2002

TYPED or PRINTED NAME Margaret Mary Kozik Richardson

REGISTRATION NO. *(if appropriate)* 47,023

Docket Number: NJMS-02-71

TELEPHONE 732-235-9350

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Complete If Known	
Application Number	
Filing Date	Herewith
First Named Inventor	HANAUSKE-ABEL, Hartmut M.
Examiner Name	
Group Art Unit	
Attorney Docket No.	NJMS-02-71

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☑ Applicant claims small entity status See 37 CFR 1.27

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FEE CALCULATION

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101	740	201	370	Utility filing fee	
106	330	206	165	Design filing fee	
107	510	207	255	Plant filing fee	
108	740	208	370	Reissue filing fee	
114	160	214	80	Provisional filing fee	80.00

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2. EXTRA CLAIM FEES

		Extra Claims		Fee from below	Fee Paid
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Independent Claims		- 3** =		X	=
Multiple Dependent					=

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103	18	203	9	Claims in excess of 20
102	84	202	42	Independent claims in excess of 3
104	280	204	140	Multiple dependent claim, if not paid
109	84	209	42	** Reissue independent claims over original patent
110	18	210	9	** Reissue claims in excess of 20 and over original patent

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FEE CALCULATION (continued)

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105	130	205	65	Surcharge - late filing fee or oath	
127	50	227	25	Surcharge - late provisional filing fee or cover sheet	
139	130	139	130	Non-English specification	
147	2,520	147	2,520	For filing a request for *ex parte* reexamination	
112	920*	112	920*	Requesting publication of SIR prior to Examiner action	
113	1,840*	113	1,840*	Requesting publication of SIR after Examiner action	
115	110	215	55	Extension for reply within first month	
116	400	216	200	Extension for reply within second month	
117	920	217	460	Extension for reply within third month	
118	1,440	218	720	Extension for reply within fourth month	
128	1,960	228	980	Extension for reply within fifth month	
119	320	219	160	Notice of Appeal	
120	320	220	160	Filing a brief in support of an appeal	
121	280	221	140	Request for oral hearing	
138	1,510	138	1,510	Petition to institute a public use proceeding	
140	110	240	55	Petition to revive - unavoidable	
141	1,280	241	640	Petition to revive - unintentional	
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122	130	122	130	Petitions to the Commissioner	
123	50	123	50	Processing fee under 37 CFR 1.17(q)	
126	180	126	180	Submission of Information Disclosure Stmt	
581	40	581	40	Recording each patent assignment per property (times number of properties)	
146	740	246	370	Filing a submission after final rejection (37 CFR § 1.129(a))	
149	740	249	370	For each additional invention to be examined (37 CFR § 1.129(b))	
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169	900	169	900	Request for expedited examination of a design application	

Other fee (specify) _____

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SUBMITTED BY				Complete (if applicable)	
Name (Print/Type)	Margaret Mary Kozik Richardson	Registration No. (Attorney/Agent)	47,023	Telephone	732-235-9350
Signature	MMRichardson			Date	10/10/2002

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Docket Number	NJMS-02-71

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Number 2 of 2

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Art Unit	:	None assigned	**Docket No.**	:	NJMS-02-71
Examiner	:	None assigned	**Dated**	:	October 10, 2002
Serial No.	:	Unknown			
Filed	:	Herewith			
Inventors	:	Hartmut M. Hanauske-Abel, et al.			
Title	:	"Ligands of Eukaryotic Translation Initiation Factor 5A (eIF-5A) and Their Use in Diagnosis and Therapy of Diseases Involving Proliferation of Cells"			

Assistant Commissioner of Patents
Washington, DC 20231
BOX PROVISIONAL PATENT APPLICATION

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October 10, 2002
Date

Ligands Of Eukaryotic Translation Initiation Factor 5A (eIF-5A) And Their Use In Diagnosis And Therapy Of Diseases Involving Proliferation Of Cells.

The government has certain rights in this invention.

Abstract

The present invention relates to ligands that specifically bind to the hypusine domain (hd) of eukaryotic translation initiation factor 5A (eIF-5A). The ligand is preferably an immunologic reagent, i.e. an eIF-5A/hd-eIF-5A binding antibody or an eIF-5A/hd-eIF-5A binding fragment, derivative, or homologue thereof. The invention provides an immunologic reagent to detect and identify proliferating cells in biological fluids and in tissues, and to measure the amount of eIF-5A, hd-eIF-5a, and their key building block hypusine in biological samples. In one embodiment, the immunologic reagent is used in an immunohistology assay or in flow cytometry. In another embodiment it is used in a quantitative immunoassay involving a radiological or chemical detection step.

In addition, where appropriately incorporated into suitable expression vectors encoding the immunologic reagent or functional equivalent, such ligands are contained in cells. Upon binding of such a ligand to eIF-5A, the biological activity of eIF-5A is modified in a manner such that the binding of eIF-5A to proteins binding eIF-5A physiologically is inhibited; the translation of eIF-5A-dependent mRNAs is inhibited; the proliferation of cells is inhibited; and the multiplication of retroviruses that rely on the host eIF-5A, in particular human immunodeficiency virus, is inhibited.

Background

The eukaryotic translation initiation factor 5A (eIF-5A) exists in two genetically distinct variants, I and II, which both contain a single hypusine residue, formed by enzymatic hydroxylation within a collagen motif of the sequence -Gly-X-Y-Gly- [Figure 1]. Hypusine is not genetically encoded. The residue derives from a genetically encoded lysine moiety, after butylamine transfer utilizing spermidine and hydroxylation utilizing atmospheric oxygen [Figure 2]. In culture, reversible suppression of hypusine formation correlates with reversible arrest in the late G1 phase of the cell cycle, immediately before the initiation of DNA replication (1).

Inhibitors of deoxyhypusyl hydroxylase (DOHH), the hydroxylating enzyme, typically cause arrest at the immediate G1/S boundary of the cell cycle jointly with the disappearance from polysomes of a unique subset of cellular mRNA's termed *hymns* (hypusine-dependent messenger nucleic acids) (2). Reactivation of DOHH causes rapid reappearance of *hymns* at polysomes and subsequent, highly synchronized entry of cells into S phase (2). The *hymns*, though encoding very diverse cell cycle-relevant proteins, share common nucleotide motifs, termed JSBs, in their untranslated 3' and 5' regions. Recent structural analyses of eIF-5A indicate that its C-terminal part folds like the cold-shock protein A of *E. coli*, which prevents mRNA duplex formation at low temperatures, and that its N-terminal part contains motifs II, III, IV, and V of ATP-utilizing mRNA helicases, required for unwinding of mRNA duplexes (3). DOHH activity is also required for the intracellular multiplication of retroviruses, in particular human immunodeficiency virus (HIV) whose genome encodes Rev, a protein specifically interacting with eIF-5A. Inhibition of DOHH activity suppresses the formation of infectious HIV virions by removing from the host cell polysomes the retroviral mRNAs encoding the capsid proteins (4).

Based on the above background, we hypothesized that ligands that bind to eIF-5A, and in particular its hypusine domain, would in tissues identify those cells that are in the process of proliferation, i.e. initiating or undergoing replication of their DNA. The identification of such ligands has multiple applications in conditions that involve cell proliferation.

Detailed Description of the Invention

It is established that the presence - or absence, respectively - of hypusine in eIF-5A, mediated by activity - or inactivity, respectively - of DOHH, correlates with the initiation - or the cessation, respectively - of proliferation of cells as well as the initiation - or the cessation, respectively - of multiplication of infectious HIV particles (1-4). Cognizant of the biological import of hypusine and the hypusine domain, we decided to generate antibodies against the structure of bioactive eIF-5A isolated from red blood cells.

Surprisingly, although of polyclonal origin the antibody we generated was entirely non-reactive with the protein as encoded by the human eIF-5A genes ('lysine precursor'), i.e. the form lacking hypusine entirely and displaying a lysine side chain instead (comp. Figures 1 - 3). The protein representing the half-product formed during post-translational modification ('deoxyhypusine precursor') was marginally reactive with the polyclonal antibody (comp. Figures 1 - 3). In contradistinction, the protein representing the final product formed by post-translational

modification, i.e. the hypusine-containing eIF-5A proper, was highly reactive with NIH-353 (comp. Figures 1 - 3). The specificity of NIH-353 for eIF-5A *only if it contains hypusine* makes this antibody a principal tool for the identification of natural and man-made molecules that are able to bind to, or otherwise interact with, with the hypusine domain of eIF-5A proper. Such identification may employ, and rely on, NIH-353 in a number of techniques, exemplified by competitive assays.

When used as a reagent in routine immunohistochemistry procedures, the NIH-353 antibody *did not* generate any distinctive staining of human tissues. Surprisingly, however, microwave antigen retrieval (MAR) performed on standard tissue slides *did* render the same NIH-353 antibody highly reactive. Only the proliferating cells in MAR-treated tissue sections were labeled by NIH-353 (comp. Figure 4). This is entirely consistent with the extensive *in vitro* data on the essential role that the hypusine domain of eIF-5A proper plays in cell proliferation.

For those knowledgeable in the art, the outlined results represent information directly enabling i) the detection of proliferating cells in histology sections; ii) the therapeutic control of eIF-5A availability as required for cell proliferation and retroviral replication; and iii) the high-throughput screening for natural and/or man-made compounds that target the hypusine domain of eIF-5A.

Examples

The examples are representative of the work and are not intended to limit the scope of the invention.

Example 1

Production of NIH-353.

Human eIF-5A protein, known to contain the hypusine domain of eIF-5A proper, was isolated as described (5). Polyclonal antiserum against purified human eIF-5A was generated in rabbits.

Example 2

Characterization of NIH-353 as specifically directed against the hypusine domain of eIF-5A.

To establish the antigen specificity of NIH-353, decreasing concentrations of the three biosynthetic forms of eIF-5A [protein as encoded by the eIF-5A gene ('lysine precursor'); protein representing the half-product formed during post-translational modification ('deoxyhypusine precursor'); and protein representing the final product formed by post-translational modification, i.e. the hypusine-containing eIF-5A proper] were studied side-by-side on Western blots, using a commercially available product (NuPage™ Bis-Tris Electrophoresis System; Invitrogen Life Technologies, Carlsbad, CA). NIH 353, the primary antibody, was diluted 1/1000 in TTBS (0.15 M NaCl, 0.01 M Tris, 0.0.05% Tween) with 0.5% milk proteins. After multiple washes in TTBS, the membrane was agitated in TTBS/0.5% milk proteins containing the secondary anti-rabbit antibody, diluted 1/40000. Signal was developed with a commercial chemoluminescence reagent (Renaissance™; NEN, Boston, MA) on commercial film (BioMax MR™; Kodak, Rochester, NY).

Example 3

Characterization of NIH-353 as selectively reacting with proliferating cells in human tissues

To establish the staining selectivity of NIH-353, we used immunocytochemical methods as published (6). Antigen retrieval was performed in a commercially available liquid (Citra™; BioGenex, San Ramon, CA), employing physical parameters that we have optimised for work with NIH-353 and involves cycled microwave irradiation at temperatures above 94.7 °C. We used the streptavidin-biotin/horseradish peroxidase complex technique, with diaminobenzidine as chromogen and hematoxylin as counterstain. Formalin-fixed paraffin-embedded human tissues were sectioned to contain at least one proliferative, anatomically defined area.

Figures

Figure 1

Binding site sequence for ligands of eukaryotic translation initiation factor 5A (eIF-5A).
Deduced amino acid sequences for the two presently known molecular variations of eIF-5A in humans, each one encoded by a distinct gene. The hypusine residue is formed from, and occurs in the position of, the labeled lysine (K*). The hypusine domain, representing the binding site sequence, is underlined.

Figure 2

Synthesis of the hypusine residue defining the hypusine domain of eIF-5A, essential for its biological function.
The genetically not encoded hypusine residue within the hypusine domain of eIF-5A is generated by a two-step post-translational modification of a genetically encoded lysine side chain. Spermidine is stoichiometrically consumed in the first, molecular oxygen in the second step. Thus, eIF-5A exists in three biosynthetic forms, two of them half-products (lysine precursor; deoxyhypusine precursor) and one of them the hypusine-containing eIF-5A proper.

Figure 3

Specificity of the eIF-5A ligand NIH-353: Interaction with only the mature variety among the three biosynthetic forms of eIF-5A.
Western blot of the lysine precursor ('Lysine'), the deoxyhypusine precursor ('Deoxyhypusine'), and of the hypusine-containing eIF-5A proper ('Hypusine'), each shown at decreasing concentrations of purified protein. NIH-353 does not interact with the lysine precursor even at 1 mg/ml, and interacts with the deoxyhypusine precursor only marginally at concentrations that must exceed 100 ng/ml. By contrast, NIH-353 avidly binds to hypusine-containing eIF-5A proper even at concentrations as low as 1 ng/ml.
MW markers, molecular weight markers.

Figure 4
Selectivity of the eIF-5A ligand NIH-353: Labeling of cells preferentially in the proliferative zones of human tissues.
Typical results obtained in human tissues are exemplified by squamous epithelium, endometrium, and endometrial surface epithelium. Staining obtained with Ki-67, a standard antibody widely used in pathology to detect proliferating cells in human tissue samples, is shown for comparison.
NIH-353 produces a signal that localizes to the cytoplasm, Ki-67 a signal that localizes to the nucleus. Thus, NIH-353 does not stain the nuclei, and Ki-67 does not stain the cytoplasm, of proliferating cells.
In the squamous epithelium, NIH-353 and Ki-67 label the proliferating cells in the basal layers.
In endometrium, NIH-353 and Ki-67 label the proliferating endometrial glands.
In endometrial surface epithelium, NIH-353 and Ki-67 label the proliferating cells but not the underlying stroma.

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Claims

We claim the following:

1. A ligand specific for the hypusine domain of eukaryotic intiation factor 5A (eIF-5A), said hypusine domain being defined as containing hypusine residues that correspond to residues 40 to 60 of the human sequence shown in Figure 1, and said ligand being characterized as binding to the hypusine domain of eIF-5A such that in biological samples said binding produces a detectable signal for identification of eIF-5A.

2. A ligand as claimed in Claim 1, in which the ligand is an antibody, or a derivative or fragment thereof, which specifically binds to an eIF-5A molecule, either because said eIF-5A contains hypusine, or if eIF-5A lacks the hypusine residue does not bind in an amount greater than 5% of the extent to which said antibody binds hypusine-containing eIF-5A.

3. An antibody, or a derivative or fragment thereof, as claimed in Claim 2, which specifically binds to a human eIF-5A molecule, only if said eIF-5A contains hypusine.

4. A method for distinguishing proliferating cells from non-proliferating cells in a specimen of biological fluid or tissue, said method comprising:

    a. Processing a specimen of biological fluid or tissue to yield a mixture of cells, said mixture of cells consisting of proliferating and non-proliferating cells as contained in the biological fluid or tissue; and
    b. Treating said mixture of cells with a fixing agent to form a sample of premeabilized fixed cells; and
    c. Reacting said sample of fixed cells with a ligand as defined in Claim 1, which specifically binds the domain containing hypusine of eIF-5A as in Figure 1; and
    d. Separating said sample from unreacted ligand as defined in Claim 1; and
    e. Detecting said ligand as defined in Claim 1 remaining within the cells of said sample, whereby detection of said ligand permits detection of said ligand involves a signal selected from the group consisting of a radiolabel, an enzyme, a chromophore and a fluorescer thereby permitting identification of proliferating cells.

5. A method as in Claim 4, additionally comprising of depositing said specimen on a solid support, and detection of said ligand within the cells of said specimen is performed using a microscope.

6. A method as in Claim 4, additionally comprising of maintaining said specimen in suspension, and detection of said ligand within the cells of said speciman is performed using a flow cytometer.

7. A method for distinguishing proliferating cells from non-proliferating cells in a specimen of biological fluid or tissue, said method comprising:

a. Processing a specimen of biological fluid or tissue to yield a mixture of cells, said mixture of cells consisting of proliferating and non-proliferating cells as contained in the biological fluid or tissue; and
b. Treating said mixture of cells with a fixing agent to form a sample of premeabilized fixed cells; and
c. Reacting said sample of fixed cells with a ligand as defined in Claim 2, which specifically binds the domain containing hypusine of eIF-5A as shown in Figure 1; and
d. Separating said sample from unreacted ligand as defined in Claim 2; and
e. Detecting said ligand, remaining within the cells of said sample, whereby detection of said ligand involves a signal selected form the group consisting of a radiolabel, an enzyme, a chromophore and a flourescer thereby permitting identification of proliferating cells.

8. A method as in Claim 7, additionally comprising of depositing said specimen on a solid support, and detection of said ligand within the cells of said specimen is performed using a microscope.

9. A method as in Claim 7, additionally comprising of maintaining said specimen in suspension, and detection of said ligand within the cells of said speciman is performed using a flow cytometer.

10. A method for distinguishing proliferating cells from non-proliferating cells in a speciman of biological fluid or tissue, said method comprising:

a. Processing a specimen of biological fluid or tissue to yield a mixture of cells, said mixture of cells consisting of proliferating and non-proliferating cells as contained in the biological fluid or tissue; and
b. Treating said mixture of cells with a fixing agent to form a sample of premeabilized fixed cells; and
c. Reacting said sample of fixed cells with a ligand as defined in Claim 3, which specifically binds the domain containing hypusine of eIF-5A as shown in Figure 1; and
d. Separating said sample from unreacted ligand as defined in Claim 3; and
e. Detecting said ligand, remaining within the cells of said sample, whereby detection of said ligand involves a signal selected form the group consisting of a radiolabel, an enzyme, a chromophore and a flourescer thereby permitting identification of proliferating cells.

11. A method as in Claim 10, additionally comprising of depositing said specimen on a solid support, and detection of said ligand within the cells of said specimen is performed using a microscope.

12. A method as in Claim 10, additionally comprising of maintaining said specimen in suspension, and detection of said ligand within the cells of said speciman is performed using a flow cytometer.

13. A method of diagnosing a hyperproliferative disorder comprising contacting a biological sample with a ligand as defined in Claim 1 and detecting said ligand bound to eIF-5A present in the sample, which is indicative of a hyperproliferative disorder.

14. A method of diagnosing a hyperproliferative disorder comprising contacting a biological sample with a ligand as defined in Claim 2 and detecting said ligand bound to eIF-5A present in the sample, which is indicative of a hyperproliferative disorder.

15. A method of diagnosing a hyperproliferative disorder comprising contacting a biological sample with a ligand as defined in Claim 3 and detecting said ligand bound to eIF-5A present in the sample, which is indicative of a hyperproliferative disorder.

16. A method of diagnosing intraepithelial neoplasia comprising contacting a biological sample with a ligand as defined in Claim 1 and detecting said ligand bound to eIF-5A present in the sample, which is indicative of local neoplasia.

17. A method of diagnosing intraepithelial neoplasia comprising contacting a biological sample with a ligand as defined in Claim 2 and detecting said ligand bound to eIF-5A present in the sample, which is indicative of local neoplasia.

18. A method of diagnosing intraepithelial neoplasia comprising contacting a biological sample with a ligand as defined in Claim 3 and detecting said ligand bound to eIF-5A present in the sample, which is indicative of local neoplasia.

19. A method of diagnosing intraepithelial neoplasia comprising contacting a biopsy containing epithelium with a ligand as defined in Claim 1 and detecting said ligand bound to eIF-5A present in the sample, which is indicative of local neoplasia.

20. A method of diagnosing intraepithelial neoplasia comprising contacting a biopsy containing epithelium with a ligand as defined in Claim 2 and detecting said ligand bound to eIF-5A present in the sample, which is indicative of local neoplasia.

21. A method of diagnosing intraepithelial neoplasia comprising contacting a biopsy containing epithelium with a ligand as defined in Claim 3 and detecting said ligand bound to eIF-5A present in the sample, which is indicative of local neoplasia.

22. A method for determining in a biological sample the concentration of eIF-5A and of hypusine, either as a free amino acid or bound within the hypusine domain that corresponds to residues 40 to 60 of the human sequence shown in Figure 1, comprising:

a. contacting said sample with a ligand as defined in Claim 1, under conditions wherein said ligand can form an immune complex with hypusine contained in the sample either as a free amino acid or bound within the hypusine domain shown in Figure 1; and
b. determining the amount of eIF-5A and of hypusine bound by said ligand by detecting the amount of immune complex formed using a signal selected from the group consisting of a radiolabel, an enzyme, or a chromophore and a flourescer.

23. A method for determining in a biological sample the concentration of eIF-5A and of hypusine, either as a free amino acid or bound within the hypusine domain that corresponds to residues 40 to 60 of the human sequence shown in Figure 1, comprising:

a. contacting said sample with a ligand as defined in Claim 2, under conditions wherein said ligand can form an immune complex with hypusine contained in the sample either as a free amino acid or bound within the hypusine domain shown in Figure 1; and
b. determining the amount of eIF-5A and of hypusine bound by said ligand by detecting the amount of immune complex formed using a signal selected from the group consisting of a radiolabel, an enzyme, or a chromophore and a flourescer.

24. A method for determining in a biological sample the concentration of eIF-5A and of hypusine, either as a free amino acid or bound within the hypusine domain that corresponds to residues 40 to 60 of the human sequence shown in Figure 1, comprising:

a. contacting said sample with a ligand as defined in Claim 3, under conditions wherein said ligand can form an immune complex with hypusine contained in the sample either as a free amino acid or bound within the hypusine domain shown in Figure 1; and
b. determining the amount of eIF-5A and of hypusine bound by said ligand by detecting the amount of immune complex formed using a signal selected from the group consisting of a radiolabel, an enzyme, or a chromophore and a flourescer.

25. A method for inhibiting in a cell the biological activity of the hypusine domain of eIF-5A that corresponds to residues 40 to 60 of the human sequences shown in Figure 1, comprising:
a. introducing into said cell of a patient in need of treatment a nucleic acid molecule encoding an antibody homologue, a derivative or fragment thereof; and
b. Said antibody homologue, a derivative or fragment thereof, specifically reactive to the hypusine domain of eIF-5A; and
c. Said antibody homologue is expressed intracellularly and binds to said hypusine domain intracellularly and thereby inhibits the biological activity of the hypusine domain of eIF-5A.

26. The method of Claim 25, wherein the antibody homologue is a single chain Fv fragment.

27. The method of Claim 25, wherein the nucleic acid molecule is a recombinant expression vector selected from the group consisting of viral vectors and plasmid vectors.

28. A method of identifying by high throughput screening a therapeutic agent that decreases the biological activity of the hypusine domain of eIF-5A, comprising contacting eIF-5A with an agent and detecting the binding of an antibody of Claim 2 to eIF-5A.

29. A method according to Claim 25, wherein the high throughput screening of the biological activity of the hypusine domain of eIF-5A is directed at cell proliferation.

30. A method according to Claim 25, wherein the high throughput screening of the biological activity of the hypusine domain of eIF-5A is directed a retroviral multiplication.

31. A method of identifying by high throughput screening a therapeutic agent that decreases the biological activity of the hypusine domain of eIF-5A, comprising contacting eIF-5A with an agent and detecting the binding of an antibody of Claim 3 to eIF-5A.

32. A method according to Claim 31, wherein the high throughput screening of the biological activity of the hypusine domain of eIF-5A is directed at cell proliferation.

33. A method according to Claim 31, wherein the high throughput screening of the biological activity of the hypusine domain of eIF-5A is directed a retroviral multiplication.

34. A method of identifying by high throughput screening a therapeutic agent that decreases the biological activity of the hypusine domain of eIF-5A, comprising contacting eIF-5A with an agent and detecting the binding of an antibody of Claim 1 to eIF-5A.

35. A method according to Claim 34, wherein the high throughput screening of the biological activity of the hypusine domain of eIF-5A is directed at cell proliferation.

36. A method according to Claim 34, wherein the high throughput screening of the biological activity of the hypusine domain of eIF-5A is directed a retroviral multiplication.

Figure 1

```
NP_001961; eIF-5AI     1  MADDLDFETGDAGASATFPMQCSALRKNGFVVLKGRPCKIVEMSTSKTGK*  50
NP_065123; eIF-5AII    1  MADEIDFTTGDAGASSTYPMQCSALRKNGFVVLKGRPCKIVEMSTSKTGK*  50

NP_001961; eIF-5AI    51  HGHAKVHLVGIDIFTGKKYEDICPSTHNMDVPNIKRNDFQLI-GIQDGYL   99
NP_065123; eIF-5AII   51  HGHAKVHLVGIDIFTGKKYEDICPSTHNMDVPNIKRNDYQLIC-IQDGYL   99

NP_001961; eIF-5AI   100  SLLQDSGEVREDLRLPEGDLGKEIEQKY--DCGEEI-LITVLSAMTEE-A  145
NP_065123; eIF-5AII  100  SLLTETGEVREDLKLPEGELGKEIEGKYNA--GEDVQV-SVMCAMSEEY-  145

NP_001961; eIF-5AI   146  AVAIKAMAK 154
NP_065123; eIF-5AII  146  AVAIKPC-K 153
```

FIGURE 2

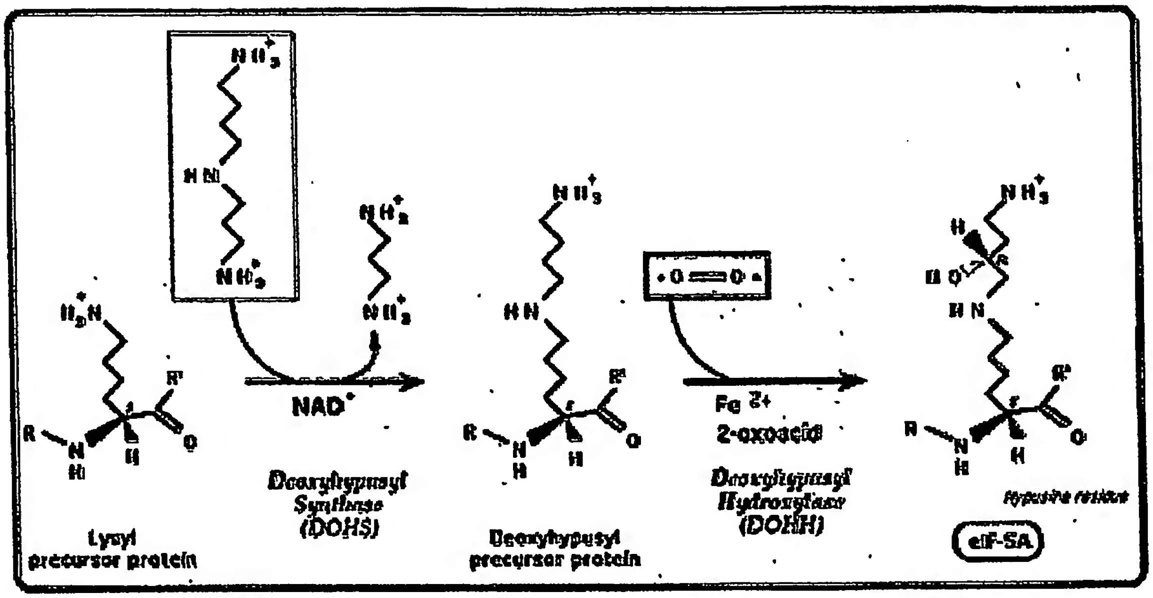

NAD+
Deoxyhypusyl Synthase (DOHS)
Lysyl precursor protein
Deoxyhypusyl precursor protein
Fe 2+
2-oxoacid
Deoxyhypusyl Hydroxylase (DOHH)
hypusine residue
eIF-5A

FIGURE 3

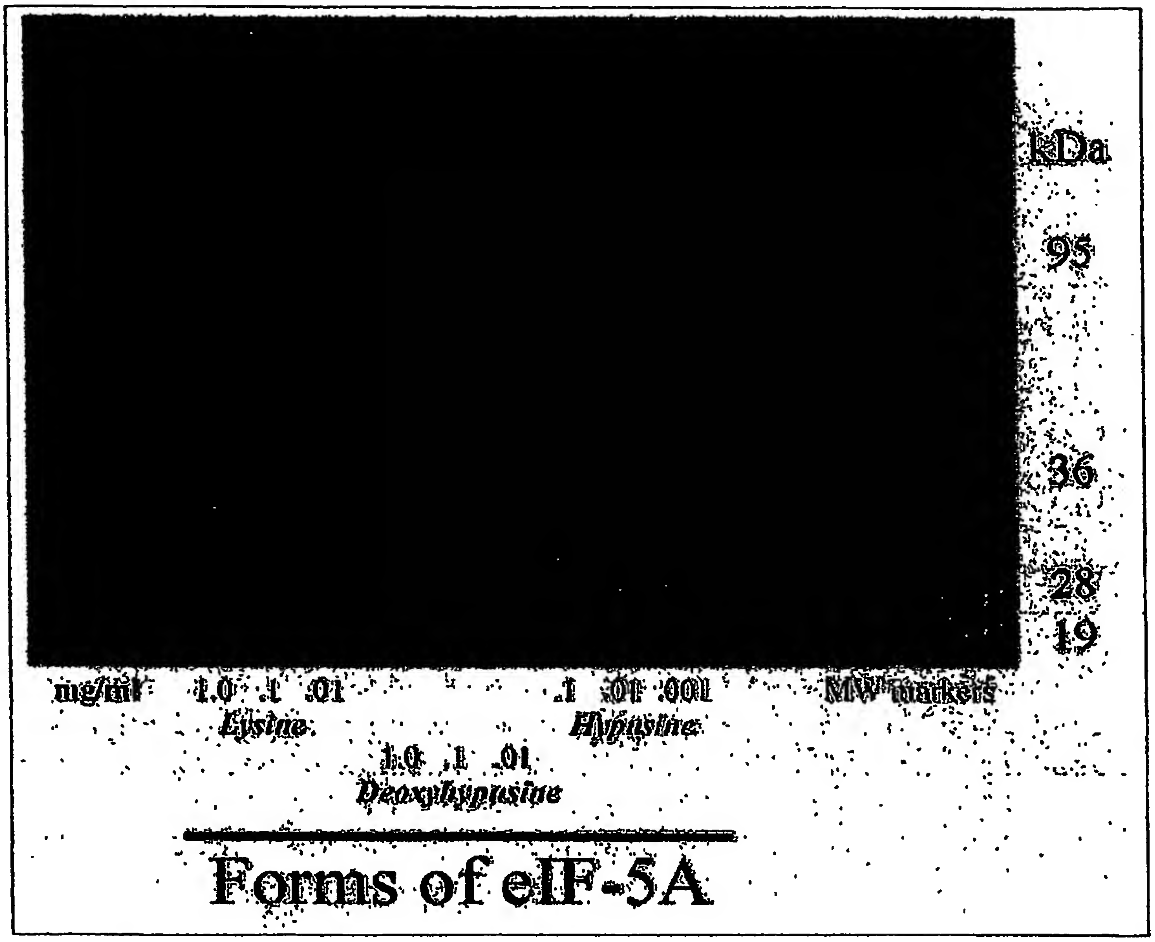
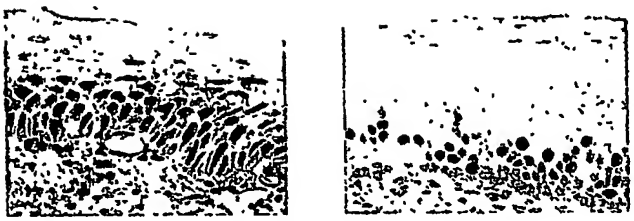
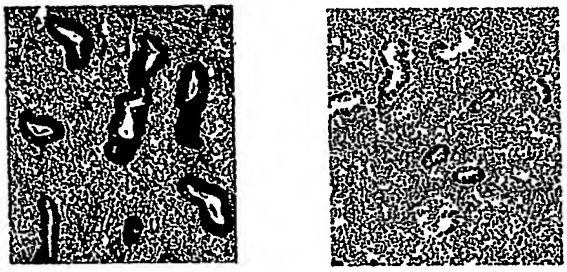

kDa
95
36
28
19
mg/ml 1.0 .1 .01
Lysine
.1 .01 .001
Hypusine
MW markers
1.0 .1 .01
Deoxyhypusine
Forms of eIF-5A

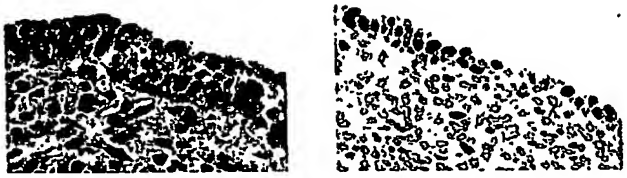
FIGURE 4



Squamous epithelium
(tonsil)



Endometrium
(proliferative phase)



Endometrium
(surface epithelium)

NIH-353 **Ki-67**